

Protocol: DNA preparation in 96 well format using seed crusher

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Reagents:

Crushing solutions
0.25 N NaOH

Neutralization
0.05 M Tris-HCL pH 7.0
0.1 mM EDTA

Flat-bottom microtiter plate (Rainin Instrument Co., R96-0APF-1CO)

Procedure:

1. Put a 96-well PCR plate on ice, add 75 μ l ice-cold Neutralization solution into each well, and cover it with the 96-well plate lid.
2. Add 7.5 μ l ddH₂O into each well of a second 96-well (flat-bottomed) plate and put it on ice.
3. Collect tissue disks of leaf tissue into the flat-bottomed 96-well plate using a hole punch.
4. Add 100 μ l of 0.25 N NaOH to each well and grind for 5 minutes (**no more than 5 min. !!!**) using a 96 well seed crusher (ISOLABS, OH). Make sure all leaf disks are at the bottom of wells.
5. Pipette 7.5 μ l of ground sample to the 96-well PCR plate containing 75 μ l of Neutralization solution.